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DNA Precipitation and Hybridization (CGH)

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Dextran sulfate (50%)
Intergen, Cat. S4030
Ethanol, absolute
Formamide, deionized
Human Cot-1 ^{TM} DNA, 1 mg/ml
GIBCO BRL, Cat. 15279-011, 500 µg
Salmon testes DNA, 9.7 mg/ml
SIGMA Molecular Biology, Cat. D-7657, 1 ml
SSC, 20X
Sodium acetate (Na-Acetate), 3M
Water, sterile

Preparation

Master Mix

Dextran sulfate, 50%	40 ml
20X SSC, pH 7.0	10 ml
Sterile H_2O	50 ml

Vortex solution and place tube on a shaking platform overnight to insure proper mixing.
Aliquot, and store at -20°C

70% Formamide/2X SSC

Deionized formamide	70 µl
20X SSC	3 µl
Sterile water	27 µl

Adjust to pH 7.5

Procedure

1. Add to an eppendorf tube:
   10-25 µl nick-translated test or tumor probe DNA (200-500 ng DNA)
   Equal amount of nick-translated control whole genomic DNA as probe DNA
   (Note: can use 1-2 µg DNA if tumor DNA is isolated from paraffin material)
   10-20 µl human Cot-1 DNA (1 mg/ml)
   8-10 µl salmon sperm DNA (10 mg/ml)

   Note: Usually the test DNA is nick translated with Biotin-16-dUTP and the control DNA is nick translated with Digoxigenin-11-dUTP.

2. Add 1/10 volume Na-Acetate (3M).

3. Add 2.5-3.0 x total volume of absolute ethanol.

4. Vortex, store at -20°C overnight, or at -80°C for at least 15-30 min.

5. Centrifuge (14000 rpm) precipitated DNA at 4°C for 30 min.

6. Pour off supernatant and speed vac for 5-10 min to dry pellet.

7. Add 6 µl deionized formamide (pH 7.5), incubate at 37°C for 30 min, shaking; vortex a few times during the 30 min incubation.

8. Add 6 µl Master Mix, vortex, and centrifuge briefly.

9. Denature probe DNA at 76°C for 5 min and centrifuge briefly. Can keep at 37°C until ready to denature.

10. Preanneal at 37°C for 1-2 hr.

11. For slide denaturation apply 120 µl 70% formamide/2X SSC to a 24 x 60 mm coverslip and touch slide to coverslip.

12. Incubate slides at 75°C for 1.5 min.

13. Immediately place in ice cold 70% ethanol for 3 min, followed by 90% ethanol and 100% ethanol for 3 min each; air dry.

14. Add probe DNA after preannealing to denaturated slides and cover with 18 mm^2 or 22 mm^2 coverslips; seal coverslips with rubber cement.

15. Hybridize at 37°C in a humidified chamber for at least 48 hr.